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The Cell Surface Hydrophobicity of *Flexibacter maritimus*

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The cell surface hydrophobicity and autoagglutination of *Flexibacter maritimus* were determined. The hydrophobic properties were compared by two different methods: salt-agglutination test (SAT) and phase partitioning with hydrocarbon solvents (BATH). *F. maritimus* GBR-3 was relatively weakly hydrophobic regardless of the method used to evaluate this capacity. Autoagglutination was not observed in the strain tested.

Key words: *Flexibacter maritimus*, cell surface hydrophobicity, fish pathogen

Flexibacter maritimus infection has been a common problem during the fry and adult stages of cultured red sea bream *Pagrus major*, black sea bream *Acanthopagrus achlegeli*, and Japanese flounder *Paralichthys olivaceus*, which are among the most economically important marine fish species in Japan.

The cell surface hydrophobic propotions of pathogenic bacteria play an important role in host cell attachment. Recent research has demonstrated that the possession of a hydrophobic wall is advantageous to pathogenic bacteria, because it allows intracellular survival and multiplication in the phagocytic cell of diseased animals (Beachery *et al.* [1]; Daly *et al.*, [2]). In this study the cell surface hydrophobicity and autoagglutination of *F. maritimus* were examined.

Materials and Methods

Bacterial strain

F. maritimus GBR-3 isolated in August 1985 from diseased red sea bream at Hiroshima Prefecture, Japan was used in this study. *Cytophaga columnaris* KM-1 isolated in 1992 from diseased Japanese eel *Anguilla japonica* at Yamagata Prefecture, Japan. *Streptococcus iniae* ATCC 19178 and *Enterococcus seriolicida* ATCC 49156 were used for the control of

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autoagglutination test.

Salt-agglutination test

The salt-agglutination test (SAT) was performed by using the method of Lindhal *et al.* [3] *F. maritimus* GBR-3 was inoculated into a liquid medium containing 0.1 % Trypton, 0.1 % casamino acid, 0.02 % yest extract, 3.13 % NaCl, 0.07 % KCl, 1.08 % $MgCl_2 \cdot 6H_2O$, and 0.1 % $CaCl_2 \cdot 2H_2O$ at pH 7.0 (TCY) in a flask and was incubated at 20 ℃ for 48 h with shaking. The culture fluid was centrifuged to collect the bacterial cells at $8,100 \times g$ for 60 min. The cells were washed twice in 13 mM phosphate buffered saline (PBS) at pH 7.0 and suspended in this buffer to an absorbance of 1.0 at 420 nm. Twenty-five microliters of this suspension was mixed with an equal volume of buffered ammonium sulphate solution that ranged in molarity from 0.05 to 5.0 M. The mixture was incubated at 20 ℃ for 12 and 24 h without shaking, then agglutination was assessed by the absence of turbidity in the mixture.

BATH test

Partitioning in liquid hydrocarbons was determined using the method of Rosenberg *et al.* [4] The bacterial strain was cultured, harvested and washed twice in PBS as described in the explanation of SAT test. Washed bacterial cells were suspended in PBS to an absorbance of 0.2, 0.6 and 1.2 at 600 nm (A_{600}). The bacterial suspensions of 1.2 m*l* were overlaid on *n*-hexadecane, *n*-octane or xylene of 0.01 to 0.8 m*l* in glass tubes. After a 2 min agitation the mixtures were allowed to separate for 10 min at 30℃. The percent of partitioning in the hydrocarbon phase was calculated using the following formula:

$$\frac{[A_{600}\text{ (original bacterial suspension)} - A_{600}\text{ (aqueous phase)}]}{A_{600}\text{ (original bacterial suspension)}} \times 100$$

Autoagglutination test

Autoagglutination of strain was determined by the method of Janda *et al.* [5] A loopful of bacterium was inoculated into a glass tube containing 5 m*l* TCY liquid medium and incubated at 20 ℃ for 24 h without shaking. Autoagglutination was assessed by the absence of turbidity in the liquid medium and the appearance of a cellular pellet at the bottoms of the tube. For the positive control, *S. iniae* ATCC 19178 was used. Sakai *et al.* [6]

reported that *S. iniae* showed high hydrophobicity in this test. Autoagglutinating property of *C. columnaris* KM-1 and *E. seriolicida* ATCC 49156 was also determined.

Results and Discussion

The results of SAT and BATH test are shown in Table 1 and Fig. 1, respectively. In the SAT, the strain GBR-3 aggregated in the range of 4.0 to 5.0 M ammonium sulphate. The percentage adherence of the strain GBR-3 to *n*-hexadecane, *n*-octane and xylene were shown to be 15, 18 and 22 %, respectively. In the BATH test, the hydrophobicity of the strain was demonstrated as moderately positive.

Table 1. Hydrophobic property of *Flexibacter maritimus* GBR-3 determined by salt-agglutination test

Conc. of ammonium sulphate (M)	Agglutination	
	Incubation time (h)	
	12	24
0.05	−	−
0.1	−	−
0.2	−	−
0.5	−	−
1.0	−	−
2.0	−	−
4.0	+	+
5.0	+	+

−, negative; +, positive.

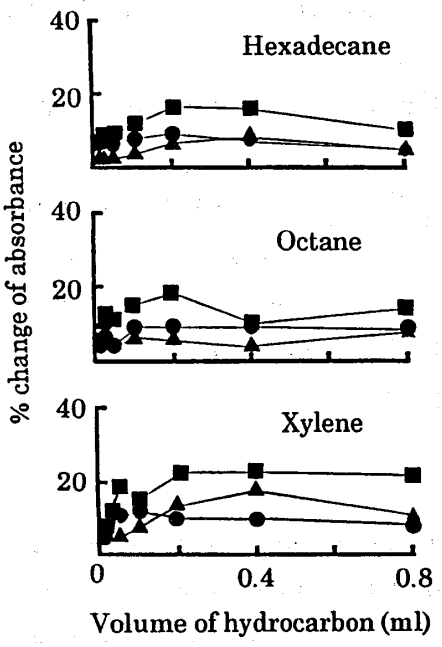

Fig. 1. Cell surface hydrophobicity of *Flexibacter maritimus* GBR-3 evaluated by BATH test.
Original bacterial suspension (A_{600}): ●, 1.2; ■, 0.6; ▲, 0.2.

Table 2. Determination of autoagglutinating property of several fish pathogen

Pathogen	Autoagglutination
Flexibacter maritimus GBR-3	−
Cytophaga columnaris KM-1	−
Streptococcus iniae ATCC 19178	+
Enterococcus seriolicida ATCC 49156	+

−, negative; +, positive.

Hydrophobicity of *F. maritimus*

The strain GBR-3 resulted in the no appearance of cellular pellet at the bottom of the tube after static culture. Thus, the autoagglutination test was negative (Table 2).

Santos *et al.* [7] investigated the hydrophobicity of bacterial fish pathogens using SAT, BATH and nitrocellulose filter binding capacity, and concluded that a single assay could not be used as a reliable representation of cell surface hydrophobicity.

In this study, the cell surface hydrophobicity of *F. maritimus* GBR-3 was determined by different methods. The results indicate that the strain GBR-3 possesses a weak hydrophobicity. The bacterial cell surface may not have intensively hydrophobic properties in host cell attachment.

Baxa *et al.* [8] reported that the pathogenicity of *F. maritimus* may be attributed to extracellular products (ECP) . Further study is needed to demonstrate the role of ECP in the pathogenic processes.

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Flexibacter maritimus 菌体の疎水性について

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海産魚の滑走細菌症原因菌 *Flexibacter maritimus* の宿主に対する付着能を明らかにするため，本菌の疎水性をSATとBATH試験によって検討するとともに，自発凝集能についても調べた。供試菌株には1985年8月に広島県で罹患マダイから分離された *F. maritumus* GBR-3株を用いた。その結果，本菌はSATでは0.05から2.0 M の硫酸アンモニウムに対しては凝集せず，4.0 から5.0 M の硫酸アンモニウムで凝集した。また，BATH試験では ヘキサデカン，オクタンおよび キシレンに対してそれぞれ最高で15，18および22 % であった。また，本菌の自発凝集能は認められなかった。したがって，疎水性は低く，自発凝集能も認められないことから，本菌の宿主に対する付着能は弱いと推察される。